Specific Determination of Sucrose in Honey

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This paper describes a procedure for determining sucrose in honey without its preliminary separation. Interference from the large excess of glucose is removed by treating with glucose oxidase-catalase, and then the glucose from invertase hydrolysis of sucrose is measured. Sucrose is difficult to measure in honey because of its low concentration and the presence of at least 24 other sugars. Standard deviation between duplicates for 50 honey samples containing from 0.06 to 10.6% sucrose was 0.19; no known honey sugars interfered. The selective adsorption method for separating sucrose from interfering oligosaccharides is too slow for repetitive analysis of honey for sucrose only, although it is 8 times more sensitive than the procedure described. Results of analysis of 13 honey samples for sucrose by this procedure (averaging 1.66%) and the selective adsorption method (averaging 1.59%) did not differ significantly.

Honey, the secretion of plant nectaries modified and stored by the honeybee, is a very complex mixture of sugars. Although glucose and fructose comprise 65–95% of the solids, the remainder includes at least 11 disaccharides and 12 higher oligosaccharides (1, 2), of which some (sucrose, isomaltose, maltose, turanose, erlose, melezitose) may be present at the 1–5% levels.

Because of its unique nature and frequently high price, honey has often been a target for adulteration. Indeed, the analytical work led by Harvey Wiley on market samples which culminated in the pure food laws of 1906 included nearly 500 samples of honey. Tests developed at that time for invert sirup and corn sirup (commercial glucose) added to honey remain in use (3).

The sucrose content of honey has been considered a measure of purity and identity since that time. The earlier official definition specified a maximum of 8% sucrose (4); this remains an informal Food and Drug Administration definition. The Codex Alimentarius (5) specifies a maximum "apparent sucrose" content of 5% for honey. (A limit of 10% is given for honeydew and for certain floral types.) The Codex requires that sucrose be determined by the increase in reducing sugar after specified mild acid hydrolysis. The nonspecificity of this procedure is acknowledged by the use of the term "apparent sucrose."

Although saccharimetric methods were used early for analysis of glucose and fructose in honey, their inadequacy was recognized by Browne (6) who used the mild acid hydrolysis for his analytical survey of United States honey. Even so, the invert polarization test still appears in *Official Methods of Analysis* (7) as an alternative to acid or enzyme hydrolysis followed by reducing sugar determination.

When the complexity of the sugars in honey became apparent, new analytical procedures were developed (8) for specific determination of glucose, fructose, and sucrose, with reducing disaccharides reported as "maltose" and higher sugars as glucose equivalent. A separation of carbohydrates by carbon column treatment into 3 size fractions precedes the analysis. This technique is an official AOAC method (9).

Sugars which interfere in the mild acid hydrolysis procedure are melezitose and erlose, both common honey constituents. Under the analytical conditions, the former appears largely in the disaccharide fraction in the column eluates; the latter is with the higher sugars. In the presence of melezitose, therefore, acid hydrolysis will give erroneously high values, and yeast invertase hydrolysis must be used (4). Erlose does not interfere, although in the absence of a class separation it is measured as sucrose by both invertase and mild acid hydrolysis.

Recently, several proposals have been made for sugar analyses of honey by gas-liquid chromatography (GLC). Pourtallier (10) and Echigo (11) used the trimethylsilyl (TMS) derivatives.

glacial acetic acid to 500 ml water, adjust to pH 4.5 with dilute NaOH, and dilute to 1 L.

(k) *Glucose oxidase-peroxidase reagent (GOP).*—Dissolve 120 mg Type II glucose oxidase and 32 mg peroxidase in 400 ml tris buffer. Add solution of 270 mg tolidine.2HCl in 520 ml water. Refrigerate in brown bottle. Filter before use if necessary.

(l) *Glucose oxidase-catalase reagent (GOC).*—Dissolve 1.00 ml Type V glucose oxidase and 0.04 ml well mixed catalase suspension in 0.01*M* phosphate buffer, pH 6.6, and dilute to 50 ml. Prepare fresh daily.

(m) *Invertase reagent.*—Dilute 12.5 mg invertase to 50 ml with 0.1*M* acetate, pH 4.5. Prepare fresh daily.

Procedure

Accurately weigh 1 g honey, transfer to 100 ml volumetric flask with ca 15 ml water, and boil on hot plate 30 sec. Cool and dilute to volume. Dilute 5.00 ml to 50 ml and pipet 5 ml of this solution into 20 × 170 mm test tube. Add 5.00 ml GOC reagent and place in 40°C water bath 1 hr. Heat 3½ min in vigorously boiling water, cool, cover, and invert to return condensate to sample. Pipet 2 ml into each of four 18 × 150 mm test tubes. Add 0.50 ml invertase to 2 tubes and 0.50 ml water to other 2 tubes. Hold at room temperature (23–26°C) 30 min.

Prepare standard as follows: Dissolve 25 mg standard glucose in 250 ml water. Either boil glucose solution 2 min before diluting to volume or hold solution 2 hr before use. Pipet 2 ml into each of 4 test tubes, and add 0.50 ml water to 2 tubes and 0.50 ml 0.1*M* acetate buffer, pH 4.5, to 2 tubes.

Place tubes in rack in order, with 1 standard tube at beginning and end and between each 2 sample tubes. Use standard with buffer added with the invertase-containing tubes, and standard with water with uninverted tubes.

Proceeding at 30 or 60 sec intervals, add 5.00 ml GOP reagent to each tube and let stand at room temperature exactly 60 min. After 60 min, add 0.15 ml 4*N* HCl to each tube, with same timing. Mix well. After 1 min, determine absorbance at 520 nm. Average absorbance of duplicate tubes and calculate glucose content of tube in micrograms by reference to average absorbance of standards on each side.

$$\text{Sucrose, \%} = 1.9\ (\mu\text{g glucose after inversion} - \mu\text{g glucose before inversion}) \times 100/(100 \times \text{mg sample})$$

Comparison with Selective Adsorption Method

A number of honey samples were analyzed with the AOAC selective adsorption (SA) method (31.124–31.126). To ensure that only sucrose was measured in fraction 2 (disaccharides), invertase hydrolysis rather than mild acid hydrolysis was applied to fraction 2 and the resulting glucose was analyzed by glucose oxidase. The same samples were also analyzed by the procedure described here. Results are shown in Table 2. Analyses of variance indicate that variance due to methods is not significant at the 5% level (Table 3). Thirty-nine samples of honey with 0.06–10.64% sucrose content were analyzed in duplicate. The standard deviation was 0.19% sucrose. The modified SA procedure is being applied to the analyses for sucrose of about 500 authentic samples of United States honey in cooperation with the Food and Drug Administration.

Table 2. Comparison of methods for sucrose in honey

SA method		New method	
Sucrose, %	Av., %	Sucrose, %	Av., %
0.40, 0.20	0.30	0.28, 0.32	0.30
5.33, 5.04	5.19	5.36, 5.00	5.18
0.50, 0.30	0.40	0.26, 0.38	0.32
0.74, 0.65	0.69	0.72, 0.93	0.82
0.90, 0.83	0.86	0.69, 0.75	0.72
1.84, 1.89	1.87	1.82, 1.83	1.82
1.95, 1.83	1.89	1.91, 1.91	1.91
0.85, 0.88	0.87	1.17, 0.99	1.08
0.61, 0.67	0.64	1.01, 0.82	0.92
0.65, 0.75	0.70	0.77, 0.98	0.87
0.72, 0.69	0.71	1.12, 0.89	1.01
0.73, 0.71	0.72	0.70, 0.70	0.70
5.87, 5.79	5.83	6.18, 5.75	5.96
Av.	1.59		1.66

In both the SA and the present method, free glucose is removed before the invertase hydrolyses to increase the accuracy of subsequent measurements. The free glucose content of the 2 ml analyzed after removal of glucose by the SA procedure averaged 4.1 μg for 10 samples. After glucose destruction in the present method, residual glucose averaged (10 samples) 11.5 μg in 2 ml. Two ml represents 8 mg honey in the SA procedure, originally containing about 2700 μg glucose. In the present method, the 2 ml analyzed represents 1 mg honey, originally containing about 340 μg glucose. The SA method is thus about 8 times more sensitive, but it is not as well adapted for analyzing large numbers of

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Battaglini and Bossi (12) analyzed 100 honey samples by GLC, quantitating only fructose, glucose, sucrose, melezitose, and 2 unidentified sugars. None of these workers eliminated the possibility that other sugar derivatives from honey cochromatographed with the sucrose derivative. Wood *et al.* (13) proposed separating the TMS derivatives of the *n*-butaneboronic derivatives but made no reference to sucrose analysis.

Hadorn and Zürcher (14), using trimethylsilyl derivatives, obtained excellent results with a model mixture of glucose, fructose, sucrose, maltose, melezitose, melibiose, and raffinose. The last 2 sugars had been tentatively reported by others as components of honey, but these investigators found none in floral honey. Analyses of 54 honey samples were reported (15). Later, Zürcher *et al.* (16), using the silylated oximes, obtained improved separation and excellent precision with mixtures of glucose, fructose, sucrose, lactose, maltose, raffinose, and melezitose. The behavior of 17 carbohydrates and derivatives was also studied. Of those of interest in honey analyses, glucose, galactose, and mannose had the same retention time, and turanose and maltose the same. Retention time of sucrose differed from all 16 sugars, but of the sugars reported in honey, only glucose, fructose, maltose, turanose, and melezitose were tested. The analysis of honey by this procedure was not reported.

The GLC analysis of honey for sucrose may well become a rapid, accurate procedure when it is established that sucrose does not share the same retention time with other sugars present in honey. At present, the only specific method available for determining sucrose in honey is the selective adsorption procedure with yeast invertase hydrolysis of the sucrose. The charcoal column separation is somewhat cumbersome if undertaken only for the sucrose determination; a method is needed to measure small amounts of sucrose in honey in the presence of melezitose and erlose (4-(α-D-glucopyranosyl)-α-D-glucopyranosyl-β-D-fructofuranoside), both of which are as easily hydrolyzed by acid as is sucrose. Table 1 lists the products of hydrolysis of these sugars by mild acid and by yeast invertase. Determination of reducing sugar before and after hydrolysis cannot be used for specific sucrose analysis of this mixture.

If yeast invertase hydrolysis is used, the specific analysis for glucose by glucose oxidase would provide specific sucrose analysis of honey, since sucrose is the only one of the 3 sugars which yields glucose. Glucose oxidase is as accurate for measuring glucose in honey as the hypoiodite oxidation used in the selective adsorption method (17).

Table 1. Hydrolysis products of honey sugars

Type of hydrolysis	Sucrose	Melezitose	Erlose
Mild acid	glucose fructose	glucose turanose	maltose fructose
Yeast invertase	glucose fructose	none	maltose fructose

The large amount of free glucose in honey makes the accurate measurement of a small increment of glucose from sucrose difficult, especially when it is considered that the average sucrose value in honey is about 1.3% (4). This paper presents a method in which most of the free glucose is destroyed by a glucose oxidase-catalase reagent before the inversion and thus allows more accurate measurement of the glucose produced by hydrolysis. This method was compared with the selective adsorption method on 13 honey samples.

Experimental

Reagents

(a) *Glucose oxidase.*—(*1*) *For glucose destruction:* Type V, repurified to remove carbohydrate hydrolases. Essentially free of maltase and invertase (Cat. No. G-6500, Sigma Chemical Co., St. Louis, MO). (*2*) *For glucose analyses:* Type II, purified (Sigma Cat. No. G-6125).

(b) *Catalase.*—From beef liver, crystalline suspension in water (Sigma Cat. No. C-30).

(c) *Peroxidase.*—Type I, from horseradish salt-free powder (Sigma Cat. No. D-8125).

(d) *Invertase.*—Grade VI, from baker's yeast, essentially melibiase-free (Sigma Cat. No. I-5375).

(e) *Tris (hydroxymethyl) aminomethane.*—Trizma base (Sigma Cat. No. T-1503).

(f) *o-Tolidine.2HCl.*—Fisher certified T-320.

(g) *Dextrose.*—Purified anhydrous glucose, NBS Sample 41.

(h) *Tris buffer.*—pH 7.6. To 48.44 g Trizma base in 500 ml water, add 384 ml 0.8*M* HCl, and dilute to 1 L.

(i) *Phosphate buffer.*—0.01*M*, pH 6.6. Add 25.2 ml 0.1*N* NaOH and 100 ml 0.1*M* KH_2PO_4 to 500 ml water, adjust to pH 6.6, and dilute to 1 L.

(j) *Acetate buffer.*—0.1*M*, pH 4.5. Add 5.72 ml

Table 3. Analysis of variance

Variance assoc. with:	Deg. of freedom	Sum of squares	Mean squares	*F* ratio	Sig., %
Methods	1	0.0340	0.0340	3.47	>5
Materials	12	78.2101	6.52	665.3	<0.1
Error	12	0.1183	0.0098		
Whole set	25	78.3624			

samples. Efforts to increase the amount of honey represented by the 2 ml aliquot analyzed were not fruitful.

It is recommended that study be continued.

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The recommendation of the Associate Referee was approved by the General Referee and by Subcommittee D and was accepted by the Association. *See* (1977) *JAOAC* **60**, 404.

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